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Chemoenzymatic Total Synthesis and Reassignment of the Absolute Configuration of Ribisin C

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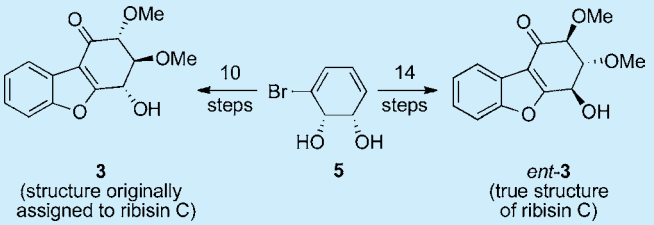
Supporting Information

ABSTRACT: The enantiomerically pure and enzymatically derived *cis*-1,2-dihydrocatechol **5** has been converted, by two related pathways, into compounds **3** and *ent*-**3**. As a result, it has been determined that the true structure of the natural product ribisin C is represented by *ent*-**3**.



Recently, Fukuyama and co-workers reported the isolation of the benzofuran-type natural products ribisins A–D (**1**–**4**, Figure 1) from *Phellinus ribis* (Schmach.) Quél. (Hymenochaetaceae),[1] a white-rot fungus found in various East Asian countries and the fruiting bodies of which are used in the traditional medicines of the region for the treatment of gastrointestinal cancer and for enhancing immunity.[2,3] The structures of the ribisins, including relative stereochemistries, were assigned on the basis of extensive and multidimensional NMR spectroscopic as well as mass spectrometric studies, while their absolute configurations were proposed based on the application of CD exciton chirality methods to the chemically derived *p*-bromobenzoates.[1] The positive and dose-dependent effects of compounds **1**–**4** on the neurite outgrowth of PC12 cells were shown to be exerted at rather low concentrations (viz. in the range of 1–30 μM) with ribisin C being the most active member of the series. As such it has been suggested[1] that these natural products could serve as useful lead candidates for the development of new treatments of certain neurodegenerative diseases.

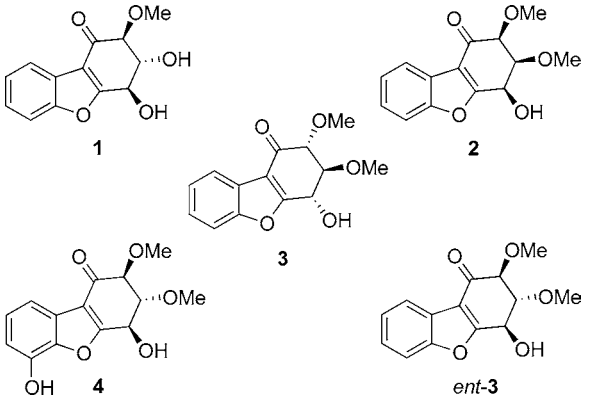


Figure 1. Ribisins A–D (**1**–**4**, respectively) and *ent*-**3**.

While a number of naturally occurring dibenzofurans have been reported,[4] the highly oxygenated nature of the ribisins and the presence of sp^3-hybridized carbons within their frameworks makes them a structurally quite distinctive group of compounds. Given such features and their intriguing biological properties we have initiated synthetic studies in the area and these have revealed, as detailed below, that the true structure of ribisin C is represented by *ent*-**3** rather than by **3** (Figure 1).[5]

The assembly of the structure, **3**, proposed for ribicin C was the initial focus of our efforts and the ultimately successful reaction sequence used for this purpose is shown in Scheme 1. Thus, the enantiomerically pure and stereochemically well-defined *cis*-1,2-dihydrocatechol **5**, which is readily obtained through the whole-cell biotransformation of bromobenzene using a genetically engineered form of *E. coli* JM109,[6] was first converted, via acetonide **6**,[7] into the well-known[7] epoxy acetonide **7**. Treatment of the last compound with aqueous hydrochloric acid resulted in the formation of the previously reported[7] *trans*-diol **8** that was subjected to a 2-fold *O*-methylation reaction using methyl iodide in the presence of sodium hydride. The acetonide moiety associated with the ensuing bis-ether **9** (90%) was cleaved with aqueous acetic acid and thus providing the *cis*-diol **10** (90%). In a pivotal step of the reaction sequence, compound **10** was subjected to Suzuki–Miyaura cross-coupling with the commercially available *o*-hydroxyphenylboronic acid pinacol ester (**11**)[8] in the presence of $PdCl_2(dppf)\cdot CH_2Cl_2$ and triethylamine and thereby affording a chromatographically separable mixture of the biaryl **12** (41%)[9] and the hoped for tetrahydrodibenzofuran **13** (24%). Product **13** presumably arises by a process in which the phenolic hydroxyl group associated with the initial cross-coupling product displaces the allylic hydroxyl group with inversion of configuration and so establishing the illustrated stereochemistry at C-4a. However, this assignment must be considered a tentative one since it is conceivable that other

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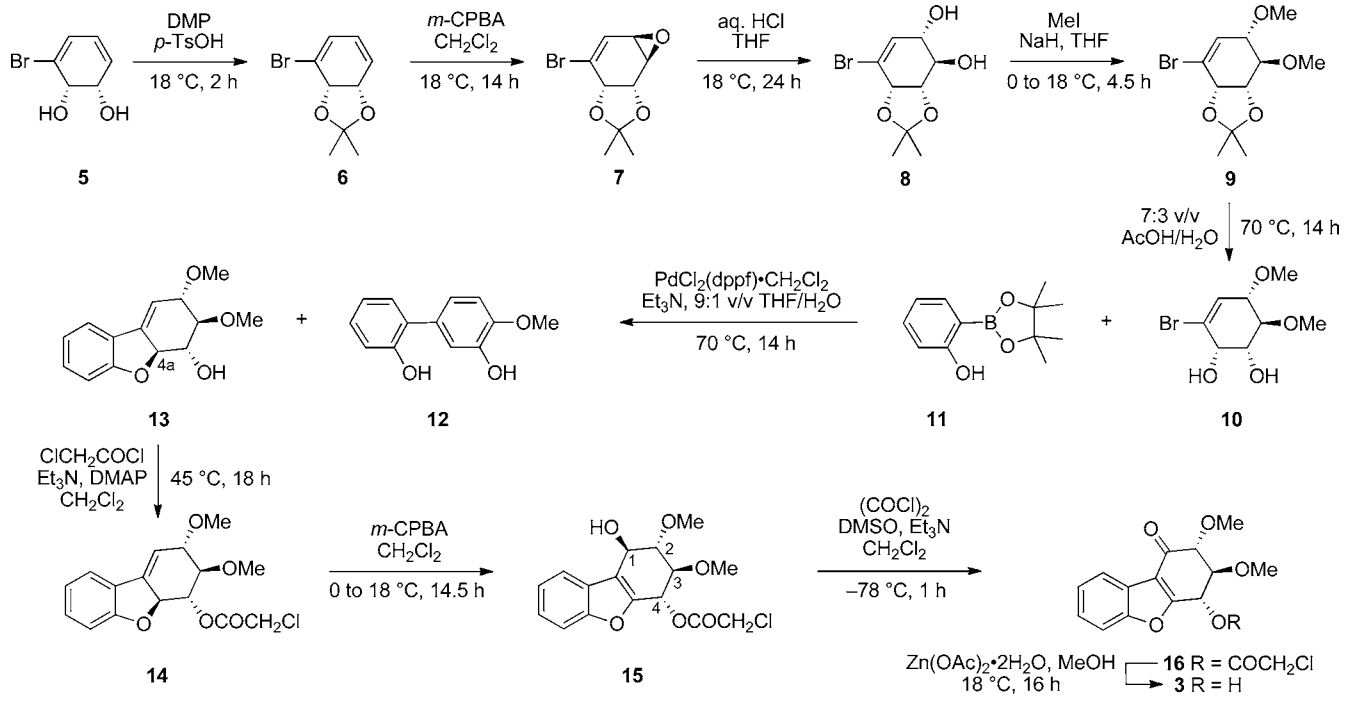
Scheme 1



cyclization pathways proceeding with retention of configuration could be involved. The precise sequence of events leading to the formation of compound **12** remains unclear at the present time but necessarily involves the loss of the elements of methanol and water at some stage.

In anticipation of the need to carry out a late-stage oxidation of a yet to be introduced hydroxyl group within the developing ribisin C framework, alcohol **13** was converted into the corresponding α-chloroacetate **14** by treating the former compound with α-chloroacetyl chloride in the presence of triethylamine and 4-(*N*,*N*-dimethylamino)pyridine (DMAP). Ester **14** was exposed to *m*-chloroperbenzoic acid (*m*-CPBA) in dichloromethane, and by such means the rather sensitive polyoxygenated tetrahydrodibenzofuran **15** was obtained as a single diastereoisomer in 49% yield (from **13**). This product presumably arises through spontaneous rearrangement of the initially formed β-epoxide, a process that is driven, at least in part, by the creation of the aromatic benzofuran substructure. The assignment of the illustrated β-stereochemistry at C-1 in compound **15** remains tentative but is based on the observation that the vicinal proton−proton couplings $J_{1,2}$ and $J_{3,4}$ observed in the 400 MHz ^{1}H NMR spectrum of this material are very similar (3.6 and 3.5 Hz, respectively) and the knowledge that a *trans*-relationship must exist between the H-3 and H-4. Subjection of alcohol **15** to Swern oxidation conditions afforded the corresponding ketone **16** (66%) that represents the α-chloroacetate derivative of the structure assigned to ribisin C. Finally, treatment of compound **16** with $Zn(OAc)_2$ in methanol at 18 °C[10,11] effected cleavage of the ester residue and thereby providing compound **3** in 71% yield. All of the spectroscopic data acquired on this product were consistent with the illustrated structure but final confirmation of it followed from a single-crystal X-ray analysis,[12] details of which are presented in the Supporting Information. A comparison (Table 1) of the ^{13}C and ^{1}H NMR spectral data acquired on the synthetically derived samples of compound **3** with those reported for ribisin C established that they were a good match. However, while the specific rotations of the two compounds were of similar magnitude they were of opposite sign[13] and thus suggesting that the absolute configuration of ribisin C had been incorrectly assigned with the true structure of this natural product being represented by *ent*-**3**.

In view of the outcomes of the studies detailed above, and because of the desire to obtain biologically active material for further evaluation, we sought to prepare compound *ent*-**3**. We were able to do so by using the reaction sequence shown in Scheme 2 and that started with the same enantiomerically pure *cis*-1,2-dihydrocatechol, **5**, as used earlier. Thus, treatment of this starting material with *N*-bromosuccinimide (NBS) in wet THF lead to a previously reported[10b] bromotriol that was immediately converted into the corresponding acetonide **17** (73% from **5**) under standard conditions. Reaction of this last compound with sodium methoxide in methanol then provided, presumably through a ring-closure/epoxide ring-opening sequence,[14] the alcohol **18** (90%) that was subjected to *O*-methylation using methyl iodide in the presence of sodium hydride and thus delivering the bis-ether **19** (97%). Hydrolysis of the acetonide residue within this last compound was readily achieved using aqueous acetic acid at 70 °C and the resulting diol **20** (93%) subjected to reaction with *p*-methoxybenzaldehyde dimethyl acetal (*p*-MBDMA) in the presence of *p*-toluenesulfonic acid (*p*-TsOH). The *p*-methoxyphenyl acetal so-formed was subjected to reductive cleavage with diisobutylaluminum hydride (DIBAl-H) and the tris-ether **21** (57%) thereby obtained.[15] Compound **21** readily participated in a $PdCl_2(dppf)\cdot CH_2Cl_2$-catalyzed Suzuki−Miyaura cross-coupling reaction with aryl boronate ester **11** in the presence of triethylamine and so delivering compound **22** (83%) as the only isolable product of reaction. Thus, while none of the anticipated cyclization product **23** was observed, this compound was readily formed, in 89% yield, by subjecting phenol **22** to an intramolecular Mitsunobu reaction using

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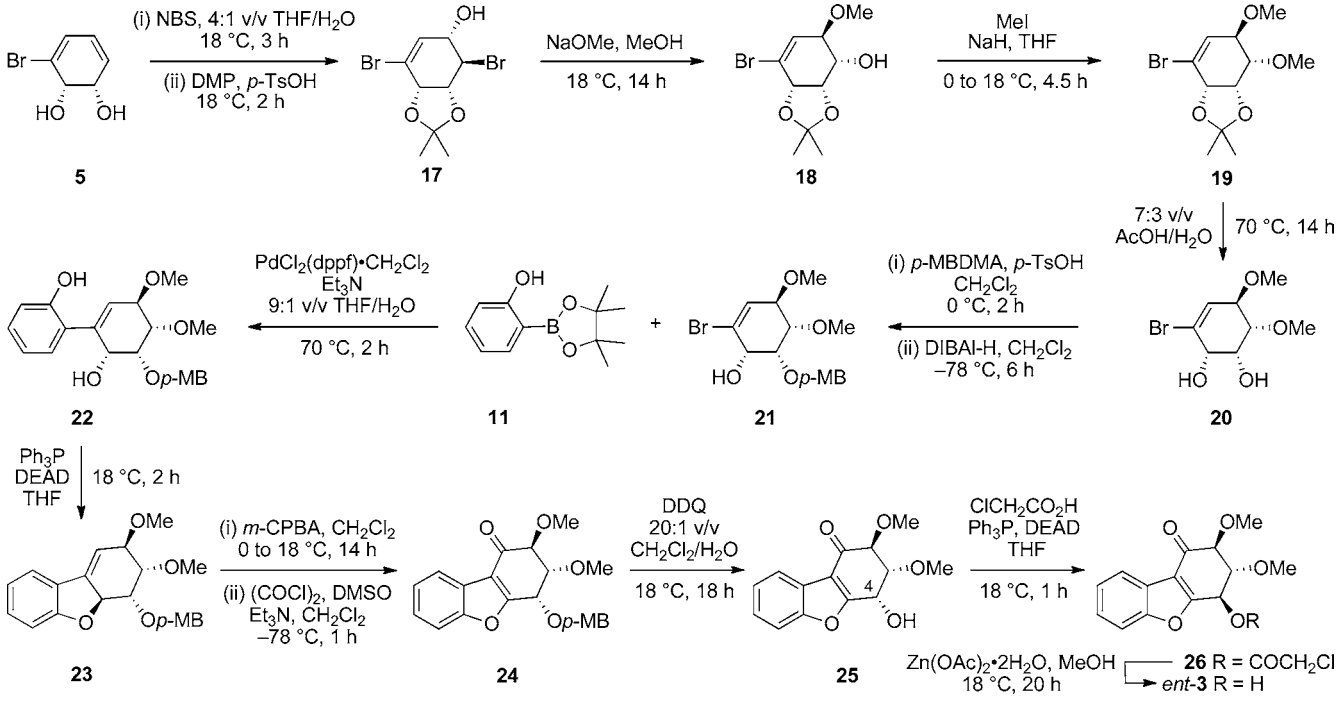
Table 1. Comparison of the ^{13}C and ^{1}H NMR Data Recorded for Synthetically Derived Compound 3 with Those Reported for Ribisin C

δ_C		δ_H	
synthetically derived compound **3**[a]	ribisin C[b]	synthetically derived compound **3**[c]	ribisin C[d]
189.9	189.9	8.07, 1H, dm, J = 7.3 Hz	8.07, 1H, ddd, J = 7.4, 1.6, and 0.6 Hz
165.9	165.9	7.56, 1H, dm, J = 7.3 Hz	7.55, 1H, ddd, J = 7.4, 1.3, and 0.6 Hz
155.7	155.7	7.42−7.34, 2H, complex m	7.39, 1H, td, J = 7.4 and 1.6 Hz
126.2	126.1		7.37, 1H, td, J = 7.4 and 1.3 Hz
124.8	124.8	4.98, 1H, ddd, J = 8.9, 4.1, and 0.9 Hz	4.99, 1H, ddd, J = 8.3, 4.3, and 0.7 Hz
123.0	123.0	4.01−3.95, 2H, complex m	3.99, 1H, dd, J = 6.1 and 4.3 Hz
122.4	122.3		3.97, 1H, dd, J = 6.1 and 0.7 Hz
114.6	114.6	3.60, 3H, s	3.61, 3H, s
111.8	111.8	3.58, 3H, s	3.59, 3H, s
83.8	83.8	3.36, 1H, d, J = 8.9 Hz	3.41, 1H, d, J = 8.3 Hz
82.8	82.9		
66.1	66.1		
59.7(4)	59.8		
59.7(3)	59.8		

[a]Data recorded in $CDCl_3$ at 100 MHz. [b]Data obtained from the Supporting Information of ref 1 and recorded in $CDCl_3$ at 150 MHz. [c]Data recorded in $CDCl_3$ at 400 MHz. [d]Data obtained from ref 1 and recorded in $CDCl_3$ at 600 MHz.

Scheme 2



triphenylphosphine (Ph_3P) in the presence of diethyl azodicarboxylate (DEAD).[16] Treatment of compound **23** with *m*-CPBA gave a rather unstable product that was immediately oxidized under Swern conditions to afford the ketone **24** in 48% yield. Cleavage of the *p*-methoxybenzyl ether (*p*-MB) residue within the latter compound was achieved using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) and thus providing C-4-*epi*-ribisin C (**25**) in 85% yield. Subjection of compound **25** to an intermolecular Mitsunobu reaction using Ph_3P/DEAD and α-chloroacetic acid as the nucleophile[10b] gave the anticipated ester **26** (84%) that could be methanolyzed in the presence of $Zn(OAc)_2$ and thereby affording the targeted and crystalline compound *ent*-**3** in 82% yield. Once again, all the NMR spectral data acquired on the latter compound were in accord with the assigned structure[17] and matched those recorded for both compound **3** and ribisin C. Significantly, the specific rotation of compound *ent*-**3** {$[\alpha]^{24}_D$ −10.8 (*c* 0.5, MeOH)} was a good match, both in terms of magnitude and sign, with that reported[1,13] for ribisin C.

The implications of the present study in terms of whether or not the absolute configurations of ribisins A, B, and D (**1**, **2**, and **4**, respectively) have also been assigned incorrectly remain unclear. It is hoped the present studies will provide the means for clarifying this matter and for generating analogues of these fascinating natural products for biological evaluation. In addition, this report further highlights the synthetic utility of the enzymatically derived *cis*-1,2-dihydrocatechols such as **5** as chiral, nonracemic starting materials for chemical synthesis.[6,18]

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■ ASSOCIATED CONTENT

Supporting Information

Full experimental procedures; ORTEPs and data derived from the single-crystal X-ray analyses of compounds **3**, *ent*-**3**, and **12** (CCDC Nos. 969616−969618, respectively); and ^{1}H and ^{13}C NMR spectra of compounds **3**, **9**, **10**, **12**, **13**, and **15**−**26**. This material is available free-of-charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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(9) The structure of compound **12** was established by single-crystal X-ray analysis. Details are presented in the Supporting Information.
(10) For other applications of these exceptionally mild ester cleavage conditions, see: (a) Saito, H.; Nishimura, Y.; Kondo, S.; Umezawa, H. *Chem. Lett.* **1987**, *16*, 799. (b) Pinkerton, D. M.; Banwell, M. G.; Willis, A. C. *Org. Lett.* **2009**, *11*, 4290.
(11) All efforts to hydrolyze the nonchlorinated analogue of ester **16** resulted in its decomposition.
(12) The absolute stereochemistry of the synthetically derived sample of compound **3** was not determined by this means but follows from the stereochemically unambiguous nature of the starting material **5** and the reaction sequence used.
(13) The optical rotation reported for ribisin C is $[\alpha]^{24}_{D}$ −11.4 (*c* 0.5, MeOH) while that obtained on synthetically derived compound **3** is $[\alpha]^{24}_{D}$ +11.1 (*c* 0.25, MeOH). It was not possible to prepare a 5.0 mg/mL solution of compound **3** in methanol because of its limited solubility in this solvent.
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